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(71) Applicant *(for all designated States except US)*: **JFS ENVIROHEALTH LTD.** [CA/CA]; 502-2871 Richmond Road, Ottawa, Ontario K2B 8M5 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LE GOFFIC, François** [FR/FR]; 42, rue Georget, F-92140 Clamart (FR). **NGUYEN, Anh, Dung** [VN/FR]; 55, Avenue du Général Leclerc, F-92340 Bourg-la-Reine (FR). **KHAYAT-FRYDMAN, Chiraz** [TN/FR]; 15 ter rue des Tournelles, F-94240 L'Hay-les-Roses (FR).

(74) Agents: **WILKES, Robert, A.** et al.; Shapiro, Cohen, 112 Kent Street, Suite 2001, P.O. Box 3440, Stn. D, Ottawa, Ontario K1P 6P1 (CA).

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(54) Title: CONCENTRATION AND PURIFICATION OF POLYUNSATURATED FATTY ACID ESTERS BY DISTILLATION-ENZYMATIC TRANSESTERIFICATION COUPLING

(57) Abstract: A process for producing highly concentrated preparations of polyunsaturated fatty acids and their esters from oils, especially fish oils, in which they occur mainly as their triglyceride esters. The process uses a combination of transesterification of the triglycerides with a lower alkyl alcohol or benzyl alcohol, molecular distillation and selective enzymatic transesterification with an alkoxy alcohol catalysed by lipases which desirably can be immobilized. The process permits the separation of the acids mixture usually encountered in the oils, and isolation of desired acids in high yields. Typical acids which can be obtained by means of this process are eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6).

CONCENTRATION AND PURIFICATION OF POLYUNSATURATED FATTY ACID ESTERS BY DISTILLATION-ENZYMATIC TRANSESTERIFICATION COUPLING

The present invention relates to a process for producing highly concentrated preparations of polyunsaturated fatty acids and their esters from oils from various sources, especially fish oils, vegetable oils and microalgae oils. The process uses a combination of molecular distillation and selective enzymatic transesterification catalysed by lipases which desirably can be immobilized.

The fatty acids mostly encountered, usually as their triglyceride esters but not exclusively, in fish oils and vegetable oils are oleic acid (C18:1), palmitoleic acid (C16:1), palmitic acid (C16:0) and gadolenic acid (C20:1), but other fatty acids possessing from 14 to 24 carbon atoms in their structure, with or without double bounds, can also be found. Polyunsaturated fatty acids possessing 3 double bonds or more are components of high economic value and have many applications in the pharmaceutical, nutrient and cosmetic fields. Two well-known families are the so-called ω3 and ω6 families, which include a single double bond located three and six carbon atoms respectively from the terminal methyl group.

The two most important members of the ω3 family are eicosapentaenoic acid (EPA, C20:5) and docosahexenoic acid (DHA, C22:6) which play an important role in human physiology. They participate in the construction of cellular membranes and, as precursors of prostaglandins, in the formation of PGI3 and TxA3, which are extremely important factors for platelet aggregation prevention. Furthermore, DHA is the most important component of brain lipids and is believed to have a role in synaptic membrane functioning.

The principal members of the ω6 family are γ-linolenic (GLA, C18:3) acid and arachidonic acid (AA, C20:4). The former is an essential fatty acid and used for certain therapeutic purposes such as the treatment of multiple sclerosis. The latter participates in prostaglandin metabolism and possesses specific functions in brain and retina.

Fish oils are important sources for EPA and DHA. In major marine oils of commerce, the weight percentages of EPA+DHA of the total fatty acids range from 7% (in Pacific herring oil) to 28% (in Pacific squid oil). But the most common source for EPA and DHA is sardine oil with a combined concentration of about 25%, comprising 18% EPA and 7% DHA. Other possible sources are salmon oil (19% EPA and DHA), menhaden oil (24%), cod liver oil (24%), and mackerel oil (15%). The ratio of EPA to DHA in fish oils is usually about 2:1, but tuna oil contains about 5% EPA and about 18% DHA. EPA and DHA are also found in lipids produced by marine microalgae such as *Monodus subterraneus* UTEX 151 (34.2% EPA), *Chlorella minutissima* UTEX 2341 (31.3% EPA), *Crypthecodinium cohnii* UTEX L1649 (19.9% DHA) or *Amphidinium carterae* UTEX LB 1002 (17% DHA).

In vegetable oils, soybean and canola oils provide γ-linolenic acid (precursor of EPA and DHA) at 8-10% of the total fatty acids. The major sources for γ-linolenic acid are borage, evening primrose and black current oils. The first is more important with 20% to 23% of γ-linolenic acid, while the second contains only about 10% of γ-linolenic acid and the last 14% to 20%.

Many methods have been proposed to obtain products enriched in polyunsaturated fatty acids using various techniques. Most have used urea complexion where the straight

chain saturated fatty acids and most monoethylenic fatty acids are removed (US 4,792,418). This process can be followed by a fractional distillation to obtain EPA in high concentration (US 4,377,526). A problem in using urea complexation is that the high quantity of urea necessary for the purification makes it difficult to produce the desired compounds on a large scale. On the other hand, the technique permits the concentration of various unsaturated fatty acids simultaneously. The composition of the reaction medium can be carefully controlled to purify selectively polyunsaturated fatty acids by the urea adduct formation method (Han, Daeseok; Shin, Hyun-Kyung; Yoon, Suk Hoo, ACS Symp. Ser. 1997, 674 (Flavor and Lipid Chemistry of Seafoods)).

EPA and DHA have also been purified through silver salt complexation (EP 454 430, and EP 303 668), by chromatography (Robles Medina,A.; Gimenez Gimenez,A.; Garcia Camacho, F.; Sanchez Perez, J.A.; Molina Grima, E.; Contreras Gomez, A.; J.Am.Oil Chem. Soc. 1995 72(5) 575-83). These processes result in the preparation of highly purified polyunsaturated fatty acids, but only in very small quantities. The silver ion, Ag^+, can be immobilized on a clay mineral (Yamaguchi, Michihiro; Tanaka, Isao; Ohtsu, Yutaka; Yukagaku 1991 40(10), 959-64), which makes the method more practical.

Supercritical extraction and liquid-liquid extraction are also alternative techniques of purification (JP 09157684 and JP 09263787).

A very promising method currently used to obtain concentrates of polyunsaturated fatty acids is molecular distillation. This method makes use of high vacuum conditions to distil the thermolabile polyunsaturated fatty acids. This process, in some cases, results in very high concentrates of

polyunsaturated fatty acids. A British patent (GB 2 218 984) has claimed the process to be appropriate for industrial production of EPA and DHA. However multiple distillations are necessary to obtain a high concentration of polyunsaturated fatty acids and the distillation temperature used is very low (from 50 to 85°C). Using a low temperature has an advantage of not altering the nature of the easily oxidized polyunsaturated fatty acids, but causes a major problem in industrial production since the throughput is proportional to the difference between the vapour pressure of the products to be distilled and the operating pressure, and the former is a function of temperature.

Although very powerful, the concept of molecular distillation is close to that of batch distillation and thus has limitations. In particular, if the composition of the raw material is complex, molecular distillation does not allow an effective purification and for instance is inappropriate for the production of DHA free from EPA and vice versa. The same problem is encountered for the production of γ-linolenic acid free from linoleic and oleic acids.

Another way to obtain polyunsaturated fatty acids in high concentration is selective hydrolysis or selective synthesis using specific enzymes. Different lipases known to have a selectivity for saturated fatty acids over polyunsaturated fatty acids are used. The most common way is to hydrolyse oils containing polyunsaturated fatty acid triglycerides followed by washing or extraction (JP 05095792, JP 03108489, and JP 07203979).

This invention seeks to provide an efficient economical process for the concentration and the purification of polyunsaturated fatty acids as monoesters of lower alcohols.

The raw material for the process is either fish oil containing different concentrations of eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6), or vegetable oils like borage, evening primrose or black current oils, containing different concentrations of γ-linolenic acid (GLA C18:3), or oils isolated from microalgae or other micro-organisms which contain fatty acids like DHA or arachidonic acid (AA C20:4). In general, these desired acids are usually present in the natural oil as the triglyceride ester.

In this invention the fatty acid triglyceride ester is first transesterified chemically. The esters are either submitted to fractionation concentration (EPA-DHA) prior to enzymatic transesterification catalysed by an appropriate enzyme, or directly enzymatically transesterified. The course of the reaction is followed by gas chromatography. It is stopped by the removal of the enzyme by filtration or centrifugation. The transesterified oil product is submitted, generally, to two molecular or so-called short path distillations.

In the first one, the excess of transesterification alcohol is removed as distillate, and the oil is also deodorized. In the second one, performed on the residue of the first distillation, a distillate is produced comprising the unsaturated fatty acid ester of interest as a generally clear, odorless compound. The residue of this distillation can also be used as a potential wax base, or split into its component fatty acids, or fatty acid esters of lower alcohols, which can also be purified by short path distillation.

In more detail, in a preferred embodiment this invention comprises a multistep process which can be summarised as follows.

Step 1. Oils containing polyunsaturated fatty acid triglycerides, such as sardine oil, borage oil and the like as described above, are first transesterified for three hours, with a primary lower alkyl alcohol at room temperature, using an alkali metal alcoholate of the same lower alkyl alcohol as catalyst. The esters obtained are separated from the glycerol formed during the reaction by decantation. The excess alcohol is removed under vacuum (15 mbars). The mixture is then treated according to its composition and to the polyunsaturated acids to be purified, using the following procedure.

Step 2. The mixture of ethyl esters is subjected to a preliminary optional short path distillation at 160-190°C and a pressure of 10^{-3} mbar to remove impurities, which constitute the distillation residue. The distillate comprises the desired fatty acid ethyl esters.

Step 3: The distillate from step 2, or the esters mixture from step 1 if step 2 is omitted, is subjected to short path distillation at 130-160°C and a pressure of 10^{-3} mbar to separate the mixture into two ester fractions. The distillate is rich in esters of fatty acids possessing 18 carbons or less, and the distilland is rich in esters of fatty acids possessing 20 carbons or more.

Step 4: The purified ethyl esters from Step 3 are transesterified with a mono- or poly-alkoxy alcohol using a reaction specific lipase, in the presence of sufficient water for the lipase enzyme to catalyse the transesterification reaction, to provide a mixture of fatty acid ethyl esters rich in polyunsaturated fatty acids and fatty acid alkoxyalkyl esters rich in saturated and monounsaturated fatty acids.

Step 5: The unreacted alcohol remaining after the transesterification reaction in Step 4 is removed by short path distillation at 60-90°C under a pressure of 10^{-3} mbar, or by washing with water followed by a suitable drying procedure.

Step 6: The esters mixture from Step 5 is subjected to a final short path distillation at 100-180°C to separate the fatty acid ethyl esters distillate from fatty acid alkoxy alkyl esters (distilland).

In the purification procedure, one or more steps can be bypassed if they are not necessary. For example, steps 1 and 2 are not used in the purification of γ-linolenic acid from borage oil as the principal constituents of this oil are linolenic, linoleic and oleic acids, which cannot be separated efficiently by distillation. Step 4 is omitted whenever a too high molecular weight alcohol such as polyethylene glycol methyl ether 350 is used.

In step 1 the primary lower alkyl alcohol preferably contains up to 7 carbon atoms, or can be benzyl alcohol. More preferably, the lower alkyl alcohol is ethyl alcohol.

In step 4 the amount of enzyme used is preferably from about 0.5% to about 5.0% by weight of the fatty acid esters present in the oil, and the amount of water present is from 1% to 10% by weight of the enzyme.

Preferably in step 3 the lipase enzyme is chosen from the group consisting of: Lipozyme IM from a *Mucor miehei* strain; Lipase D from a *Rhizopus delemar* strain; and Esterase 30000 from a *Mucor miehei* strain.

Preferably, the transesterification in step 4 is carried out at a temperature of 50 - 70°C, and a pressure of from about 0.1 - 20 mbars. More preferably, the transesterification in step 4 is carried out at a temperature of 60 - 70°C, and a pressure of about 15 mbars.

The present invention provides a process to obtain polyunsaturated fatty acids on a large scale. It can be applied to different kinds of oils by coupling short path distillation and selective enzymatic transesterification. The two techniques can be used in a modular way in order to obtain products with a desirable composition. The combination makes it possible for the following to be obtained:

(a) products containing the desired fatty acids at very high concentrations which may reach 90% or higher in many cases, even with complex starting materials;

(b) products containing tailor-made compositions,

(c) very high yield and very high throughput in the purification sequence of steps;

(d) the purification process does not require the use of a solvent; and

(e) it is possible to separate EPA from DHA.

In any of the purification steps, whenever the product is subject to heating, for either a distillation step or in the enzymatic reaction, it is always under vacuum. This minimises any oxidation and the degradation of the thermolabile polyunsaturated fatty acid esters.

The polyunsaturated fatty acids are purified as ethyl esters, whose vapour pressure is higher than that of the corresponding free acids. This makes it possible to obtain a high throughput in the short path distillation steps at 130-180°C of up to 150 $kg/m^2.h$.

Steps 1 and 2 are used principally for the purification of EPA and DHA. The ethyl esters of these fatty acids have a much lower rate of distillation than that of lower fatty acids (C18, C16 and lower). Thus a preliminary distillation makes it possible to obtain products containing up to 33% EPA and 22% DHA, and eliminates a high proportion of the lower fatty acids which are also present. The quantity of products to be treated enzymatically is therefore much smaller than the original material, usually from about 10% to about 20% of the starting material, according to its composition.

The key step of the whole procedure is the transesterification of the fatty acid ethyl esters with a mono - or polyalkoxy alcohol, such as 2-(2-butoxyethoxy)ethanol and polyethylene glycol methyl ether 350, using a reaction specific lipase such as Lipozyme IM (Novo) under a vacuum of 10-20 mbars, and at appropriate temperature, such as 60-70°C for Lipozyme). The reaction equilibrium is displaced by removing under vacuum the ethanol formed during the reaction. The rate at which the enzyme catalyses the transesterification reaction depends on the degree of unsaturation of the fatty acid in the ethyl ester; the reaction generally becomes slower as the degree of unsaturation increases. Consequently, it is found that saturated and monounsaturated fatty acid ethyl esters are first transesterified, and the transesterification of polyunsaturated fatty acid ethyl esters takes place generally at a much lower rate. There remains however a difference of behaviour between EPA and DHA which is not recognized by the lipases, and EPA reacts relatively slowly. After the transesterification process, a mixture of fatty acid ethyl esters rich in polyunsaturated fatty acids and fatty acid alkoxyalkyl esters rich in saturated and mono unsaturated fatty acids is obtained.

After the transesterification step, the remaining excess alcohol can be suitably removed by short path distillation. As they possess usually a higher vapour pressure than that of the fatty acid esters, it is easy to separate them from the latter by a short path distillation at 60-90°C at a pressure of 10^{-3} mbars; under these conditions the distillation rate of the fatty acid esters is insignificant. Thus the alcohol is recovered as the distillate, and can be recycled.

The fatty acid ethyl esters rich in polyunsaturated fatty acids are then separated from the products of the enzymatic reaction comprising the fatty acid alkoxyalkyl esters rich in saturated and monounsaturated fatty acids by a second short path distillation at 100-180°C under a pressure of 10^{-3} mbars. The fatty acid ethyl esters rich in polyunsaturated fatty acids are recovered in the distillate while the fatty acid alkoxyalkyl esters rich in saturated and monounsaturated fatty acids, whose vapour pressure is practically nil, are recovered as the distilland. This short path distillation step is also used to remove the excess of alcohol if it has a zero vapour pressure, as in the case of polyethylene glycol.

The distillate product obtained is highly rich in polyunsaturated fatty acid ethyl esters, and can contain more than 80% in most cases and can reach 90% or more.

The choice of alkoxy alcohol is crucial for the purification. The alkoxy alcohol esters formed by the enzymatic reaction cannot be distilled in the range of temperature used for the distillation of fatty acid ethyl esters and thus the two ester types can be separated very efficiently by short path distillation. The enzyme selectivity is much higher with an alkoxy alcohol than with an aliphatic alcohol. With oleyl alcohol, for example, the enzymatic

transesterification by Lipozyme IM is practically the same for all the fatty acids while with polyethylene glycol methyl ether 350, the reaction rate for saturated and monounsaturated fatty acids esters is at least 12 times greater than that for EPA (C20:5) ester and DHA ester. This characteristic allows not only the enrichment of polyunsaturated fatty acids but also the separation of DHA from EPA. The mixture of fatty acid ethyl esters enriched in EPA and DHA is subject to a transesterification with polyethylene glycol methyl ether by Lipozyme IM to convert EPA into its ester with polyethylene glycol methyl ether, which is then separated from DHA ethyl ester by short path distillation. EPA is recovered in its free form after a saponification or hydrolysis, or as an ester of a lower alcohol by a suitable transesterification reaction. γ-linolenic acid, which is one of the polyunsaturated fatty acids in borage and evening primrose oils, can also be isolated by the above procedure. 2-(2-butoxyethoxy)ethanol seems to be the best alcohol for the purification of γ-linolenic acid ester, as γ-linolenate ethyl ester with a concentration of up to 90% can be obtained at a yield of up to about 96%.

The present invention will now be illustrated by the following examples. In these examples, all analysis data was obtained by suitable chromatography procedures.

Example 1.

To 3 litres of absolute ethanol, 23 g of sodium are added. When all the sodium is dissolved, 10 kg of sardine oil, containing 18% EPA, and 7% DHA as triglycerides, is added. After 3 hours of reaction under agitation at ambient temperature and nitrogen atmosphere, the mixture is decanted, and the glycerol separated at the bottom of the mixture is removed. The ethyl esters solution is then dried and degassed under vacuum (10 mbars). About 10 kg of products is recovered.

The product is then submitted to short path distillation at 160 °C under a pressure of 10^{-3} mbars, and about 9.5 kg of clear, limpid ethyl esters is obtained as the distillate, containing 18% EPA and 7% DHA as their ethyl esters.

The ethyl esters mixture is then submitted to short path distillation at 150 °C under a pressure of 10^{-3} mbars. The distilland representing 15 to 30% of the throughput is recovered. This product contains at least 28% to 40% EPA, and 20% to 35% DHA, as the ethyl esters. With more careful control, the process can produce 33% EPA and 22% DHA, as the ethyl esters.

Example 2.

1000 parts by weight of fatty acid ethyl esters containing 33% EPA and 22% DHA obtained by the process of Example 1 is transesterified with 550 to 900 parts by weight of polyethylene glycol methyl ether MW 350 and 10 to 20 parts by weight of Lipozyme IM in one presence of sufficient water for the enzymatic reaction to proceed.. The reaction is carried out under vacuum at a pressure of 10 mbars over 24 hours at a temperature of 60°C. After the reaction is complete, the enzyme is separated by filtration and can be recycled if desired. The reaction product is submitted to short path distillation at 180°C under a pressure of 10^{-3} mbars. The distillate containing the unreacted fatty acid ethyl esters is recovered. Total concentration of EPA and DHA ethyl esters in this distillate is between 75 and 82% (EPA from 35 to 38%, DHA from 40 to 44%).

Example 3.

1000 parts by weight of fatty acid ethyl esters containing 35% EPA and 40% DHA from Example 2 is transesterified with 700 to 850 parts by weight of polyethylene glycol methyl ether MW

350 and 20 to 50 parts by weight of Lipozyme IM in one presence of sufficient water for the enzymatic reaction to proceed under vacuum at a pressure of 10 mbars for 48 hours at a temperature of 60°C. After transesterification, the enzyme is separated, and the product is distilled by short path distillation at 180°C under a pressure of 10^{-3} mbars to obtain a distillate containing more than 90% DHA and less than 5% EPA, and a distilland containing more than 50% EPA. 100 parts by weight of the distilland is treated with 30 parts by weight of absolute ethanol containing 0.68 parts by weight of sodium ethanolate for 3 hours under nitrogen. The product is then washed with water and dried, to give a mixture of fatty acid ethyl esters containing more than 50% EPA and less than 2% DHA.

Example 4.

1000 parts by weight of fatty acid ethyl esters containing 20% DHA (from *Crypthecodinium cohnii*), 600 to 800 parts of 2-(2-butoxy ethoxy)ethanol, and 4 to 10 parts of Lipozyme IM are stirred and heated at between 50 and 60°C under a vacuum of 9 to 15 mbars for a few hours. The reaction is stopped by removing the enzyme by filtration, and then the oil is submitted to two short path distillations. The first one is performed between 50°C and 60°C and removes excess alcohol as well as odour, and the second one is at 100-120°C; both are carried out at 10^{-3} mbar. The distillate is collected and analysed by gas chromatography. A concentrate of docosahexaenoic acid ethyl ester which can reach 95% is obtained.

Example 5.

100 parts by weight of fatty acid ethyl esters containing 40% AA (arachidonic acid; C20:5), 600 to 800 parts of 2-(2-butoxy ethoxy)ethanol, and 4 to 10 parts of Lipozyme IM, in one presence of sufficient water for the enzymatic reaction to

proceed, are stirred and heated between 50°C and 60°C under a vacuum of 9 to 15 mbars for 2 to 8 hours. The reaction is stopped by removing the enzyme by filtration. The resulting oil is submitted to two short path distillations. In the first one, which is carried between 50°C and 60°C removes excess 2-(2-butoxy ethoxy)ethanol as the distillate. The distilland from this first distillation is distilled again at 100-120°C and gives rise to an odourless distillate. Both distillations are carried out a pressure of 10^{-3} mbars. Analysis by gas chromatography reveals that a concentration of AA ethyl ester higher than 95% can be reached.

Example 6.

100 parts by weight of fatty acid ethyl esters containing 23% γ-linolenic acid (from borage oil) is transesterified with 600 to 800 parts by weight of 2-(2-butoxy ethoxy)ethanol and 6 to 10 parts of Lipozyme IM or lipase D from *Rhizopus delemar* or esterase 30000 from *Mucor mihei* strains, in one presence of sufficient water for the enzymatic reaction to proceed, under a vacuum of 9 to 15 mbars and at a temperature of 50-60°C. The reaction is stopped by removing the enzyme by filtration. The resulting oil is submitted to two short path distillations. In the first one, performed between 50°C and 60°C, excess alcohol is removed as distillate. The distilland residue thus obtained is subjected to a second distillation at 100-120°C. Both distillations are carried out at a pressure of 10^{-3} mbar. The distillate is recovered and analysed by gas chromatography. A clear odourless oil is obtained, which contains no less than 50% γ-linolenic acid ethyl ester. This concentration can reach 90%.

Example 7.

A mixture of fatty acid ethyl esters from evening primrose oil is treated under the same conditions as in Example 4.

After a few hours (between 2 and 4), the reaction is stopped and the oil treated as in Example 4. The product comprises a γ-linolenic acid ethyl ester concentrate containing between 25% and 40%. This concentration can be dramatically increased when longer reaction times are applied.

What is claimed is:

1. A process for the separation and recovery of long chain fatty acids, including mono-and polyunsaturated fatty acids, derived from at least one natural oil as the triglyceride esters thereof, which process comprises at least the following steps:

(i) subjecting the oil to a transesterification reaction with a lower alkyl alcohol in the presence of an alkali metal alcoholate of the same lower alkyl alcohol as catalyst under an inert atmosphere at room temperature, and recovering the thus formed ethyl esters;

(ii) subjecting the ethyl esters from step (i) to short path distillation at 130-160°C and a pressure of 10^{-3} mbar to separate the mixture into two fractions comprising a distillate which is rich in lower alkyl esters of fatty acids possessing 18 carbons or less, and a distilland which is rich in esters of fatty acids possessing 20 carbons or more;

(iii) subjecting the fraction from step (ii) containing the desired purified fatty acid lower alkyl ester to a transesterification reaction with a mono- or poly-alkoxy primary alcohol catalysed by a reaction specific lipase, to provide a mixture of fatty acid ethyl esters rich in polyunsaturated fatty acids and fatty acid alkoxyalkyl esters rich in saturated and monounsaturated fatty acids;

(iv) removing the unreacted mono- or poly-alkoxy alcohol remaining after the transesterification reaction in step (iii)by either short path distillation at 60-90°C under a pressure of 10^{-3} mbar, or by washing with water followed by drying;

(v) subjecting the esters mixture from step (iv)to short path distillation at 100-180°C to provide two fractions, comprising the fatty acid lower alkyl esters as distillate, and the fatty acid alkoxy alkyl esters as distilland; and

(vi) recovering the desired fatty acid from the relevant fraction from step (v).

2. A process according to Claim 1 further including between steps (i) and (ii) subjecting the mixture of lower alkyl esters to a short path distillation at 160-190°C and a pressure of 10^{-3} mbar to provide two fractions comprising a distillate which contains the desired fatty acid ethyl esters, and a distilland which is rejected.

3. A process according to Claim 1 in which the desired fatty acid is γ-linolenic acid derived from borage oil in which step (ii) is omitted.

4. A process according to Claim 1 wherein the lower alkyl alcohol is ethanol.

5. A process according to Claim 1 wherein the alkali metal in the alkali metal lower alkyl alcoholate is sodium.

6. A process according to Claim 1 wherein in step (i) the inert atmosphere comprises nitrogen.

7. A process according to Claim 1 wherein in step (iii) a monoalkoxy alcohol is used.

8. A process according to Claim 7 wherein the mono alkoxy primary alcohol has the general formula $R-O-(CH_2)_n-CH_2OH$, where n is 1, 2 or 3 and R includes 1 to 18 carbon atoms and is chosen from an alkyl group, and an alkyl group including one or two aromatic rings.

9. A process according to Claim 1 wherein in step (iii) a polyalkoxy alcohol is used.

10. A process according to Claim 9 wherein the polyalkoxy primary alcohol has the general formula $R\text{-}O(CH_2\text{-}CH_2O)_n\text{-}CH_2CH_2OH$, in which n is from 0 to 10 and R includes 1 to 18 carbon atoms and is chosen from an alkyl group, and an alkyl group including one or two aromatic rings.

11. A process according to Claim 1 wherein the lipase enzyme is chosen to catalyse preferentially the transesterification of esters of saturated or mono unsaturated fatty acids.

12. A process according to Claim 11 wherein the lipase enzyme is chosen from the group consisting of: Lipozyme IM from a *Mucor miehei* strain; Lipase D from a *Rhizopus delemar* strain; and Esterase 30000 from a *Mucor miehei* strain.

13. A process according to Claim 1 wherein the enzyme is used either as a crude preparation or immobilized on a suitable support.

14. A process according to Claim 1 wherein step (iii) is carried out by stirring the alkyl ester preparation, the alcohol and the enzyme under vacuum of 0.1 mbars to 20 mbars. and at a temperature from 50°C to 70°C.

15. A process according to Claim 1 wherein the primary lower alkyl alcohol contains up to 7 carbon atoms, or is benzyl alcohol.

16. A process according to Claim 1 wherein the amount of water is from 1% to 10% by weight.

17. A process according to Claim 1 wherein in step (iii) the weight ratio of alkyl ester to be transesterified and alkoxy alcohol is from 4:1 to 1:1.

18. A process according to Claim 1 wherein in step (iii) the amount of enzyme used is between 0.5% to 5% by weight of the esters to be transesterified.

19. A process according to Claim 15 wherein the primary lower alcohol is ethanol.

INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/CA 00/00643

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C67/03 C07C69/52 C07C69/587 C12P7/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, FSTA, COMPENDEX, BIOSIS, CHEM ABS Data, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHIMADA, ET AL.: "Enrichment of Ethyl Docosahexaenoate by Selective Alcoholysis with Immobilised Rhizopus delemar Lipase" JOURNAL OF FERMENTATION AND BIOENGINEERING., vol. 84, no. 2, 1997, pages 138-143, XP000946079 SOCIETY OF FERMENTATION TECHNOLOGY., JP ISSN: 0922-338X page 138, column 1, paragraph 2 -page 139, column 1, paragraph 4 --- -/--	1,3,4,6, 11-13, 15,16,19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
26 September 2000	11/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rooney, K

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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